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β-Fibrinogen Allele Frequencies in Peruvian Quechua, a High-Altitude Native Population

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ABSTRACT Elevated hematocrits, which are found in many high-altitude populations, increase the oxygen-carrying capacity of blood and may represent an adaptation to hypoxic environments. However, as high hematocrit increases blood viscosity, which in turn is associated with hypertension and heart disease, it may be advantageous for high-altitude populations to limit other factors that contribute to increased blood viscosity. One such factor is the plasma concentration of the coagulation protein fibrinogen. Several common polymorphisms in the β-fibrinogen gene have been identified that affect fibrinogen concentrations. We determined the allele frequencies of three of these polymorphisms (G/A^{-455}(*Hae*III), C/T^{-148}(*Hind*III), and G/A^{+448}(*Mnl*I)) in sample groups drawn from three populations: Quechua-speaking natives living at over 3,200 m in the Peruvian Andes, North American natives (Na-Dene) from coastal British Columbia, and Caucasian North Americans. The frequencies of the alleles previously shown to be associated with increased fibrinogen levels were so low in the Quechuas that their presence could be accounted for solely by genetic admixture with Caucasians. Frequencies in the Na-Dene, a Native American group unrelated to the Quechua, were not significantly different from those in Caucasians. Am J Phys Anthropol 109:181–186, 1999.

Elevated hematocrits are a characteristic response to high-altitude hypoxia in humans (Garruto, 1976). This is true in both acclimated low-landers and in high-altitude natives such as the Quechua-speaking people who inhabit the Andean altiplano in South America. Reported hematocrit values in male Quechuas living at over 3,000 m often exceed 50% (Garruto and Dutt, 1983; Winslow et al., 1989; Arnaud et al., 1985), substantially higher than Quechuas residing at lower altitudes (e.g., 42.1% in Quechua males living at 450 m; Arnaud et al., 1985). Elevated hematocrits increase the oxygen-carrying capacity of blood and thereby facilitate oxygen transport in low PO_2 environments. However, increased hematocrits also result in elevated blood viscosity which in turn has been associated with numerous cardiovascular disorders, including myocardial infarction and hypertension (Dintinfass, 1985). In extreme cases, excessive polycythemia can lead to chronic mountain sickness (Monge's disease), for which the only long-term cure is descent to lower altitudes (Winslow and Monge, 1987).

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Blood viscosity is also affected by the concentration of various plasma proteins, especially the clotting factor fibrinogen, which is a major determinant of plasma viscosity (Lowe et al., 1993). Fibrinogen levels are genetically regulated, and several polymorphisms affecting these levels are located in, or near, the gene encoding β-fibrinogen, one of the three polypeptides that comprise fibrinogen and the rate-determining component in holoprotein assembly (Yu et al., 1984). A guanine (G) to adenine (A) transition at base -455 (sometimes reported as being at base -453) is associated with an ~10% increase in fibrinogen in both A/A homozygotes and A/G heterozygotes (Thomas et al., 1991). A second upstream mutation, a cytosine (C) to thymine (T) transition at base -148, is associated with a similar increase (+10% in T/T homozygotes, +6% in heterozygotes; Heinrich et al., 1995). Within the gene, a G to A transition at base +448, which substitutes an arginine residue for a lysine (Schmelzer et al., 1988), has been associated with a 33% increase in plasma fibrinogen concentration in pooled homozygotes and heterozygotes (Carter et al., 1997). The phenotype varies (some studies, such as Conner et al. (1992), found no significant effect) and appears to be influenced by gender (Carter et al., 1997; de Maat et al., 1995) and by environmental conditions such as exercise (Montgomery et al, 1995). The alleles associated with increased fibrinogen are fairly common in most of the populations tested thus far, ranging from 11% in the Inuit to over 30% in some Caucasian groups (de Maat et al., 1995).

We hypothesized that selective pressure in high-altitude populations to ameliorate the detrimental effects of high hematocrit on blood viscosity might be reflected in a low frequency of the alleles associated with increased fibrinogen relative to populations residing at, or near, sea level. To test this hypothesis, we determined the β-fibrinogen genotypes of 60 Quechua-speaking natives from the Peruvian altiplano. For comparative purposes we measured the frequency of the same alleles in 50 Na-Dene from Western Canada and 31 North Americans of Caucasian extraction.

MATERIALS AND METHODS

Population and samples

Blood samples (n = 52) and buccal samples (n = 8) were obtained with informed consent from Quechua living in three communities (Huilloc, Patacancha, and Qqelcqanqa) located between 3,200–4,200 m above sea level near Cuzco, Peru. Relatedness was established by interview or by surname comparison, and first-degree relatives were excluded from the analysis. Blood was collected in 20-ml syringes and transferred into Vacutainer tubes containing either Na-Citrate or EDTA (Becton-Dickinson, Franklin Lakes, NJ). This step proved to be essential as, at 4,200 m, the pressure differential between ambient and the interior of the Vacutainer tube was insufficient to draw blood effectively. Buccal samples were obtained as described in Spitz et al. (1996). Lymphocytes were prepared from the blood samples by hypotonic cell lysis. DNA was isolated from the lymphocytes and the buccal cells by phenol/chloroform extraction. DNA was similarly prepared from blood samples obtained from 50 nonrelated North American Natives (Na-Dene) from British Columbia, Canada (Monsalve et al., 1998) and from 31 nonrelated North American Caucasians of European descent.

Genotyping

Polymorphisms in the β-fibrinogen gene were identified by restriction endonuclease digestion of polymerase chain reaction (PCR)-amplified DNA. In addition, the genotype for a *Rsa*I polymorphism in the H19 gene was determined in a subset of the Quechua as a test of heterogeneity. Primer sequences are given in Table 1. Primers Fib-B6 and Fib-B7 were previously described (Baumann and Henschen, 1993a). All primers were prepared by the University of British Columbia Nucleic Acid-Protein Service Unit. The primers in H19 were a gift from Dr. C. Brown (Department of Medical Genetics, University of British Columbia) and were previously described (Jinno et al., 1995). DNA (50–200 ng) was amplified in a Perkin Elmer (Norwalk, CT) DNA Thermal Cycler using 0.033 nmoles of each primer

TABLE 1. Primer pair sequences used for PCR amplification of regions encompassing polymorphisms in the β-fibrinogen gene

Primer	Sequence	Product size	Polymorphism
Fib-1C	5′ AAGGGTCTTTCTGATGTGTA 3′	204 bp	G/A^{-455} (*Hae*III)
Fib-1Arev	5′ CACTAAAATCGTGACTCATT 3′		
Fib-1A	5′ ATTGAGTCACGATTTTAGTG 3′	339 bp	C/T^{-148} (*Hin*dIII)
Fib-1B	5′ GAAATGGTTACCTTTCCTTA 3′		
Fib-B6	5′ GGGACATGGCAAAGCATGG 3′	314 bp	G/A^{+448} (*Mnl*I)
Fib-B7	5′ GGTGAGCAAGAGAAATGAAG 3′		

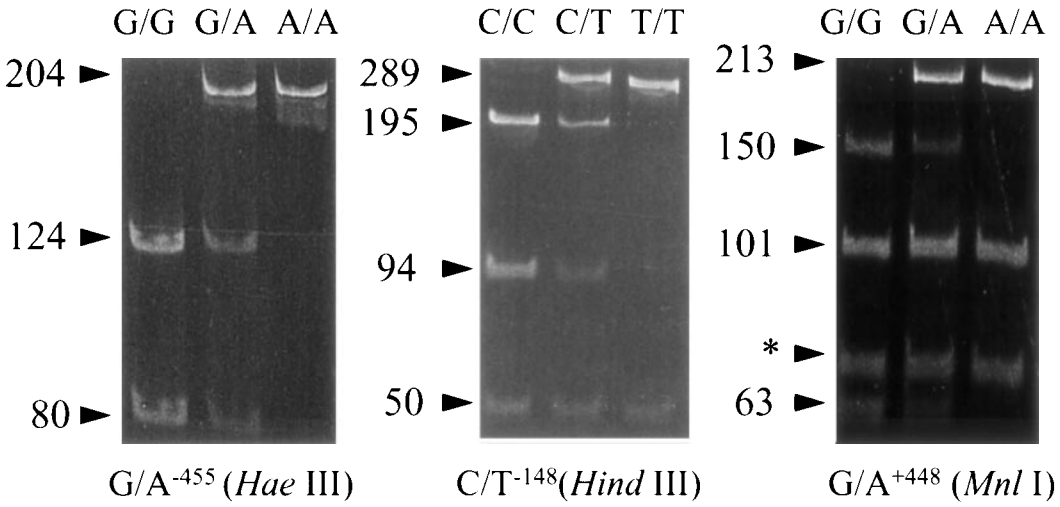

Fig. 1. Representative polyacrylamide gels, showing separation of digestion products for the RFLPs used to identify alleles of the β-fibrinogen gene. Genotypes are given along top. The polymorphism and the restriction endonuclease used to characterize it are given along bottom. *Primer artifact that did not interfere in the determination of genotype. Fragment sizes are in base pairs.

and 0.625 units *Taq* polymerase (Gibco BRL, Gaithersburg, MD). The final reaction mixture (25 µl) was 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 20 mM Tris/Cl, pH 8.4, and 50 mM KCl. Amplification conditions were 94°C, 1 min; 58°C, 1 min; and 72°C, 2 min for 40 cycles. Amplified product (10 µl) was then digested using 0.2–1.0 units of the appropriate restriction endonuclease under the conditions prescribed by the manufacturer (Gibco BRL, Gaithersburg, MD; NEB, Beverly, MA). Digests were electrophoresed on 8% polyacrylamide gels, stained with ethidium bromide, and recorded using a Polaroid (Cambridge, MA) photo-documentation system. Sample digests are shown in Figure 1. For data presentation, photographs were digitized using an Arcus II (Agfa, Mortsel, Belgium) scanner and incorporated into Adobe Photoshop (San Jose, CA) images.

Allele frequencies were established by gene counting and compared on 2 × 2 contingency tables by chi-square (χ^2) analysis incorporating the Yates correction for continuity (Crow, 1986).

RESULTS

Genotype and allele frequencies for the three β-fibrinogen polymorphisms in the Quechuas, Na-Dene, and Caucasians are given in Table 2. In the Quechuas, the allele frequencies for all three polymorphisms differed significantly from those of both the Na-Dene and the Caucasians ($P < 0.05$ for 1 df). The frequencies in the Caucasian sample were not significantly different from those of the Na-Dene or from those previously reported in the literature for Caucasians (e.g., Thomas et al., 1994; Montgomery et al., 1995; Carter et al., 1997). Genotype

TABLE 2. β-fibrinogen allele frequencies for the Quechua, Na-Dene, and Caucasian samples

	Quechua		Na-Dene		Caucasians	
Polymorphism	Genotypes	Allele frequency[1]	Genotypes	Allele frequency[2]	Genotypes	Allele frequency
G/A^{-455} (*Hae*III)	58 G/G	G 0.98	37 G/G	G 0.84	21 G/G	G 0.84
	2 G/A	A 0.02	10 G/A	A 0.16	10 G/A	A 0.16
	0 A/A		3 A/A		0 A/A	
C/T^{-148} (*Hind*III)	58 C/C	C 0.98	37 C/C	C 0.84	21 C/C	C 0.84
	2 C/T	T 0.02	10 C/T	T 0.16	10 C/T	T 0.16
	0 T/T		3 T/T		0 T/T	
G/A^{+448} (*Mnl*I)	58 G/G	G 0.98	37 G/G	G 0.84	22 G/G	G 0.85
	2 G/A	A 0.02	10 G/A	A 0.16	9 G/A	A 0.15
	0 A/A		3 A/A		0 A/A	

[1] The Quechua are significantly lower than both the Caucasians and the North American natives (Na-Dene) ($P < 0.05$ for 1 df).
[2] The North American (Na-Dene) native frequencies are not significantly different from those of Caucasians ($P < 0.05$ for 1 df).

frequencies were in Hardy-Weinberg equilibrium in all three samples and, with a single exception in the Caucasian group, genotypes were consistent with the haplotypes $G^{-455}/C^{-148}/G^{+448}$ and $A^{-455}/T^{-148}/A^{+448}$. The allele frequencies for the *Rsa*I polymorphism in the H19 gene in a subset of the Quechuas (n = 14) was 0.64 (T):0.36 (C).

DISCUSSION

We found the frequency of β-fibrinogen alleles A^{-455}, T^{-148}, and A^{+448} to be very low in our Quechua sample compared to our Na-Dene or Caucasian samples. As these alleles are associated with higher levels of fibrinogen, their underrepresentation may serve to mitigate the deleterious effects of the chronic polycythemia characteristic of high-altitude populations by limiting the contribution of fibrinogen to blood viscosity. Reduced fibrinogen levels would be particularly beneficial in these populations, as the effects of high fibrinogen levels on blood viscosity (Mayer et al., 1966) and on erythrocyte aggregation (Tanahashi et al., 1989) are exacerbated by high red blood cell counts. High fibrinogen concentrations and, to some extent, fibrinogen genotype have been implicated in the development of cardiovascular disease (Heinrich and Assmann, 1995; Carter et al., 1997). A paucity of the alleles associated with higher concentrations of fibrinogen could contribute to the relatively low incidence of systemic hypertension (Heath and Williams, 1995) and heart disease (Way, 1976) reported in the Quechua.

Initially, we were concerned that the limited variation at the β-fibrinogen locus that we observed in the Quechua could be due to genetic homogeneity in our sample population. To address this possibility, we determined the frequency of a common polymorphism in H19, a gene that encodes a functional RNA expressed in the fetus and placenta (Zhang and Tycko, 1992) and is nonsyntenic to the β-fibrinogen gene. The subset of Quechua DNAs that we analyzed was heterogeneous for this polymorphism, suggesting that the low frequency of a subset of β-fibrinogen alleles in our Quechuas was not simply due to genetic homogeneity. Additionally, we demonstrated heterogeneity at the angiotensin-converting enzyme locus for the same sample population (Rupert et al., 1999).

Each of the alleles associated with increased fibrinogen was quite rare in our Quechua sample (two copies in 120 chromosomes). Assuming that Caucasian admixture in our sample was similar to that previously reported for contemporary Quechuas (0.247, Salzano and Callegari-Jacques, 1988) and a frequency of the less common alleles in Caucasians of 0.16, we would expect approximately four rare alleles in our sample due to genetic admixture alone. This raises the possibility that these alleles are a relatively recent addition to the Quechua gene pool and were not present in the pre-Columbian lineage.

Both our data for the Na-Dene and data published for the Inuit (de Maat et al., 1995)

suggest that the β-fibrinogen allele frequencies that we observed in the Quechua are not characteristic of native North Americans in general. It is possible that selection has acted on the Quechua, subsequent to their original migration into the highlands, to reduce the frequencies of the alleles associated with higher fibrinogen so as mitigate the deleterious effects of elevated hematocrits on blood viscosity. This issue, however, cannot be addressed without comparing the Quechua to closely related, low-altitude control populations. As the ancestors of both the Na-Dene and the Inuit are believed to have arrived in North America during different migration waves from that which included the Quechua antecedents (Cavalli-Sforza et al., 1994), these two groups are genetically quite distant from the Quechua, and significant differences in allele frequencies due to stochastic forces are to be expected.

The mechanisms by which polymorphisms affect β-fibrinogen synthesis are unknown. Both upstream mutations are in the putative 5′ regulatory region of the gene and have been reported to alter protein:DNA interactions in vitro, suggesting that they may affect transcriptional regulation (Baumann and Henschen, 1993b; Lane et al., 1993). All three polymorphisms have been reported to be in strong linkage disequilibrium in various populations (de Maat et al., 1995; Carter et al., 1997; Thomas et al., 1994; this study), making it difficult to correlate any single allele with an observed phenotype. It is possible that there is an additive effect, and that phenotype is determined more by haplotype than by individual alleles. However, as both our data and those of de Matt et al. (1995) are consistent with complete linkage disequilibrium in Native Americans, this may not be a source of phenotypic variation in these populations.

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